Experimental Transmission of Venezuelan Equine Encephalomyelitis Virus by a Strain of *Aedes albopictus* (Diptera: Culicidae) from New Orleans, Louisiana

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ABSTRACT Experimental studies were undertaken to ascertain the vector competence of a strain of *Aedes albopictus* (Skuse) collected in New Orleans, LA, (Gentilly strain) for an epizootic (Trinidad donkey) strain of Venezuelan equine encephalomyelitis (VEE) virus. This strain of *Ae. albopictus* was significantly more susceptible to infection with VEE virus than were any of the four strains tested previously, including two from North America and two from South America. Likewise, dissemination (148 of 180) (82%) and transmission (40 of 88) (45%) rates were significantly higher in the Gentilly strain than in any of the strains previously tested. Analysis of the results of the present study along with those of a previous study with a second alphavirus, chikungunya (CHIK) virus, indicated that, although all three strains of *Ae. albopictus* tested were more susceptible to VEE virus than to CHIK virus, susceptibility to infection and dissemination with one alphavirus appeared to be directly related to susceptibility to infection and dissemination with the other virus and may indicate shared receptor sites for these two alphaviruses in *Ae. albopictus*.

KEY WORDS Insecta, *Aedes albopictus*, vector competence, Venezuelan equine encephalomyelitis virus

THE RECENT INTRODUCTION OF *Aedes albopictus* (Skuse) into the Americas has raised a concern that these mosquitoes may serve as a vector for indigenous as well as exotic viruses (Knudson 1986). Laboratory studies have demonstrated the ability of this species to transmit numerous arboviruses, including some native to the Americas (Shroyer 1986, Hawley 1988). In addition, *Ae. albopictus* has displaced populations of *Aedes aegypti* (L.) in the southern United States (Rai 1991) and has demonstrated an ability to flourish in tree holes as well as in artificial containers (Hawley 1988).

Venezuelan equine encephalomyelitis (VEE) virus, of the genus *Alphavirus*, family Togaviridae, has caused sporadic epizootics of severe disease. This disease occurs primarily in Central America, and infection is usually fatal in horses and occasionally so in man (Walton & Grayson 1989). Epizootics have occurred as far south as Ecuador and Peru in northern South America and as far north as southern Texas in 1969–1972.

A comparison of several North and South American strains of *Ae. albopictus* for their ability to transmit VEE virus under laboratory conditions indicated that, although all strains tested were competent vectors, none was a particularly efficient vector. North American strains, however, were significantly less efficient vectors of VEE virus than were South American strains, with transmission rates (after oral exposure) of 5 and 24% for the North and South American strains, respectively (Beaman & Turell 1991). However, a recent evaluation of 10 strains of *Ae. albopictus* for their susceptibility to infection with chikungunya (CHIK) virus, another member of the genus *Alphavirus*, indicated that a strain from New Orleans, LA (Gentilly strain), was the most susceptible (Turell et al. 1992a). Therefore, we evaluated the potential for this *Ae. albopictus* strain to transmit VEE virus. We also compared the relative susceptibility of selected strains of *Ae. albopictus* for VEE and CHIK viruses to determine if susceptibility to one alphavirus was related to susceptibility to a second alphavirus.

Materials and Methods

Mosquitoes. The Gentilly strain of *Ae. albopictus*, obtained from J. Freier, Centers for Disease Control, was derived from specimens collected

Research was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the "Guide for the Care and Use of Laboratory Animals," (NIH publication 86-23, 1985 ed.). The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

Table 1. Infection, dissemination, and transmission rates by day of extrinsic incubation in the Gentilly strain of *Ae. albopictus* after ingestion of $10^{4.5}$ PFU of Venezuelan equine encephalomyelitis virus (combined data from two infectious feeding trials)

Criteria	Day of extrinsic incubation					
	7	14	21	28	35	Total
Number tested	40	40	40	30	30	180
Infection rate	78%	95%	83%	83%	87%	85%
Dissemination rate[a]	73%	93%	78%	83%	87%	82%
Dissemination (I)[b]	94%	97%	94%	100%	100%	97%
Transmission rate, %[c]	50 (8)	70 (20)	38 (26)	40 (15)	32 (19)	45 (88)

[a] Percentage of all mosquitoes with virus in their legs.
[b] Percentage of infected mosquitoes with virus in their legs.
[c] Percentage transmitting (total number feeding, including uninfected mosquitoes).

in the Gentilly suburb of New Orleans, LA. They were used in the F_7 generation of colonization. Mosquitoes were maintained at 26°C using procedures described by Gargan et al. (1983); female mosquitoes were 4–10 d old when used for infection trials.

Virus and Virus Assay. A second BHK cell culture passage of an infectious clone (V3000) of the epizootic VEE subtype 1A Trinidad donkey strain (Davis et al. 1989) was used throughout these studies. This clone is biologically similar to the parent Trinidad donkey strain and has similar pathogenicity in mice, hamsters, and guinea pigs (Davis et al. 1991).

Serial 10-fold dilutions of specimens were tested for infectious virus by plaque assay on Vero cell monolayers as described by Gargan et al. (1983), except that the second overlay, containing neutral red, was added 2 (rather than 4) d later.

Determination of Vector Competence. Mosquitoes were allowed to feed on one of two anesthetized female Syrian hamsters that had been inoculated intraperitoneally 48 h earlier with 0.2 ml of a suspension containing $10^{3.5}$ plaque-forming units (PFU) of VEE virus. Immediately after feeding, three engorged mosquitoes from each hamster were individually triturated in 1 ml of diluent (10% fetal bovine serum in Medium 199 with Hanks' salts and antibiotics), frozen at −70° C, then thawed and assayed on Vero cell monolayers to determine the amount of virus ingested. The remaining engorged mosquitoes were placed in two 3.8-liter cardboard containers (one per hamster) with netting on one end. Apple slices or a 7% sucrose solution was provided as a carbohydrate source, and an oviposition substrate was added 4 d after the infectious blood meal. At 7-d intervals after the infectious blood meal, transmission attempts were made by allowing a sample of mosquitoes to feed on susceptible hamsters. On the day 7 trial, mosquitoes were tested either individually or in pools of three mosquitoes each, whereas in all other trials (days 14, 21, 28, and 35), all mosquitoes were tested individually. Immediately after each transmission trial, mosquitoes were cold-anesthetized, their legs and bodies triturated separately in 1 ml of diluent, and frozen at −70° C. Infection was determined by the recovery of virus from the mosquito body tissue samples at ≥7 d after the infectious blood meal. If virus was recovered from both body and leg suspensions, the mosquito was considered to have a disseminated infection (Turell et al. 1984). Because infection with VEE virus is virtually 100% fatal for hamsters, hamster death was used as the criterion for viral transmission. Isolation of virus from liver or brain tissue samples, or both, verified transmission. Any hamster that survived 21 d after being fed upon by a mosquito with a disseminated infection was challenged with $10^{3.5}$ PFU of VEE virus to determine its immune status.

Results

Mosquitoes ingested an average of $10^{4.5}$ PFU of VEE virus during each of the two infectious feedings. Because infection, dissemination, and transmission results were nearly identical for the two feedings, these data were combined for further analysis and are presented in Table 1. The Gentilly strain of *Ae. albopictus* was highly susceptible to infection with VEE virus, and infection rates at each of the time intervals tested did not differ significantly ($\chi^2 = 5.2$, df = 4, $P = 0.26$) from the mean of 85% (153 of 180). Dissemination rates were also high, with 97% of the infected mosquitoes having a disseminated infection. Again, there was no significant (Fisher's exact test, $P = 0.57$) association between virus dissemination and period of extrinsic incubation for specimens tested at 7–35 d after virus exposure. Likewise, no consistent difference in transmission rates was observed by time after the infectious blood meal (days 7–35) ($\chi^2 = 7.1$, df = 4, $P = 0.13$). Overall, 40 (45%) of 88 refeeding mosquitoes transmitted VEE virus.

Discussion

The Gentilly strain of *Ae. albopictus* was highly susceptible to infection with VEE virus, and 45% (40 of 88) of the orally exposed mosqui-

toes transmitted virus by bite when allowed to refeed 7–35 d later. Although strains of *Ae. albopictus* derived from specimens collected in both North and South America previously were shown to be competent vectors of VEE virus (Beaman & Turell 1991), transmission was relatively inefficient; 5% (5 of 93) and 6% (4 of 72) for the two North American and 23% (21 of 92) and 24% (19 of 78) for the two South American strains tested. The Gentilly strain was more susceptible to infection, had greater dissemination rates, and transmitted VEE virus more efficiently than did any of the strains tested in the study by Beaman & Turell (1991), even though viremia levels in the present study were ≈4-fold lower than the average of those used in the previous study. The hamster viremias to which mosquitoes were exposed in these studies are comparable with those observed in burros (Gochenour et al. 1962) and in horses (Kissling et al. 1956, Sudia et al. 1971b) inoculated with an epizootic strain of VEE virus; therefore, our results are based on viremias to which mosquitoes could be exposed in nature.

The transmission rate of VEE virus we observed for the Gentilly strain, 45% (40 of 88), is similar to the 33% (6 of 18) reported for *Psorophora confinnis* (Lynch Arribalzaga) (Sudia et al. 1971b), and the 38% (29 of 77) for *Ae. sollicitans* (Skuse) (Turell et al. 1992b). Both of these species have been implicated as natural vectors of epizootic VEE virus (Sudia et al. 1971a) and, of all the mosquito species tested, are among the most efficient transmitters for an epizootic strain of VEE virus (Sudia et al. 1971b).

To determine if susceptibility to one member of the genus *Alphavirus* was related to susceptibility to another member of this genus, we compared the relative susceptibility of the Gentilly strain of *Ae. albopictus* with published data for several other strains of this species for both VEE and CHIK viruses (Beaman & Turell 1991, Turell et al. 1992a). Although all three strains (Gentilly, Houston, and Sao Paulo) of mosquitoes tested against both viruses were more susceptible to VEE virus than to CHIK virus, susceptibility to infection and dissemination with one alphavirus appeared to be directly related to susceptibility to infection and dissemination to the other virus (Table 2). The Gentilly strain had higher infection rates than any of the other strains for both viruses, and the relative susceptibility to infection for the three strains of *Ae. albopictus* was the same for the two viruses. Likewise, dissemination rates were significantly higher in the Gentilly strain than in any of the others for both viruses (χ^2 = 56.0 and 37.4, df = 2, $P < 0.001$ for VEE and CHIK viruses, respectively), and the same relative order was maintained for both viruses (Table 2). However, the greater susceptibility of the Brazilian strains than the Houston strain to both VEE and CHIK viruses does not imply greater susceptibility to all arboviruses. For example, the Houston strain was more susceptible to all four serotypes of dengue viruses than were Brazilian strains of *Ae. albopictus* (Miller & Ballinger 1988).

Table 2. Susceptibility of selected strains of *Ae. albopictus* to infection and dissemination with Venezuelan equine encephalomyelitis or chikungunya viruses

Strain	Infection rates[a]		Dissemination rates[b]	
	VEE virus[c]	CHIK virus[d]	VEE virus[c]	CHIK virus[d]
Gentilly	85 (180)	47 (171)	82 (180)	32 (171)
Sao Paulo	62 (168)	32 (129)	50 (168)	20 (129)
Houston	54 (127)	20 (176)	39 (127)	6 (176)

[a] Percentage infected (number tested).
[b] Percentage with a disseminated infection (number tested).
[c] Gentilly data from present study; Sao Paulo and Houston data from Beaman & Turell (1991).
[d] Data for CHIK virus from Turell et al. (1992a).

The Gentilly strain was significantly more susceptible to infection with both VEE and CHIK viruses than were any of five other American strains of *Ae. albopictus* tested (Beaman & Turell 1991, Turell et al. 1992a). In addition, strains of *Ae. albopictus* collected in the Gentilly area of New Orleans differed significantly in photosensitivity, susceptibility to freezing, and in their susceptibility to infection with *Dirofilaria immitis* from *Ae. albopictus* collected elsewhere (G. Craig & G. Scoles, unpublished data). Although these differences may be the result of founder effects during the dispersal of *Ae. albopictus* across the United States, they are also indicative of multiple introductions. Multiple introductions of this species have almost certainly occurred, as evidenced by the interdiction of 11 tires infested with *Ae. albopictus* (Craven et al. 1988). Based on their study, they estimated that >2,000 tires containing *Ae. albopictus* larvae were imported into the United States from Asia alone during 1985–1986. Regardless of whether the Gentilly strain represents a separate importation of a potentially more efficient vector or merely the product of a founder effect, the great susceptibility of this strain to two alphaviruses increases the risk of transmission of these arboviruses by *Ae. albopictus* within the United States.

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